Conformational Changes of Deoxyribonucleic Acid and Polydeoxynucleotides in Water and Ethylene Glycol*

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ABSTRACT: Further experiments are reported here in an attempt to elucidate the structure of DNA in ethylene glycol. We have studied four different DNAs and a number of polydeoxyribonucleotides of defined structure with respect to their optical rotatory dispersion, circular dichroism, and absorption spectra as well as the changes in these parameters as a function of temperature. All DNA samples showed the same qualitative behavior, with certain quantitative differences perhaps related to their guanine-plus-cytosine content. Based on the properties presented none of the synthetic deoxyribopolymers studied can be considered a wholly satisfactory model for DNA. We have also compared the deoxyribopolymers and relevant ribopolymers both in water and ethylene glycol and suggestions are presented concerning possible conformations.

Finally the data are examined in connection with the recent proposal that the structure of DNA in glycol corresponds to the C conformation.

Some time ago we reported that DNA assumed a novel conformation in ethylene glycol (for simplicity's sake called glycol in what follows). This conclusion was based on its anomalous and nonconservative circular dichroism and resultant optical rotatory dispersion in this solvent (Green and Mahler, 1968). In a subsequent paper (Green and Mahler, 1970) we published an extensive study comparing a number of polyribonucleotides and mononucleotides in aqueous and glycolic solutions with the objective of trying to draw analogies between these biosynthetic polymers and DNA. We showed that the polyribonucleotides in glycol exhibit rotatory spectra that do not resemble the DNA spectrum in the same solvent. A possible reason for this dissimilarity could be related to the basic chemical difference between the two classes of polynucleotides, namely, the presence or absence of a 2′-OH group.

In order to answer this question, we have carried out an analogous study on the polydeoxynucleotides and now wish to report the results. This permits us to compare the behavior of the polymers not only with that of DNA, but also with that of the corresponding polyribonucleotides and thus draw certain inferences about similarities and differences with regard to their conformation in solution.

While some of the deoxyribopolymers resemble DNA in certain of their optical parameters more closely than did the ribopolymers, none can be regarded as a wholly satisfactory analog of DNA, as regards its conformation in solution.

Materials and Methods

DNA Samples. The DNA samples were obtained from the following sources. Calf thymus DNA was from Worthington Biochemicals, Freehold, N. J., lot no. 995. Its optical properties have been previously described (Mahler *et al.*, 1964). *Escherichia coli* K-12 DNA was from General Biochemicals, Chagrin Falls, Ohio, lots 650349 and 651799. Its properties have also been discussed (Mahler *et al.*, 1968). The *Clostridium perfringens* Type IX and salmon sperm DNA were products of Sigma Chemical Co., St. Louis, Mo. Their lot numbers were 17B-1670 and 16B-7310, respectively.

Synthetic Polydeoxyribonucleotides. $(dA)_n$ and $(dT)_n$[1] were gifts from Dr. Fred Bollum, University of Kentucky, Lexington, Ky. Their properties have been previously described (Ts'o *et al.*, 1966). The duplex, $(dA)_n \cdot (dT)_n$ was prepared by mixing equimolar concentrations of the two homopolymers. The concentrations of the homopolymers were determined using the molar extinction coefficients of Table I. $(dAdT)_n \cdot (dAdT)_n$ was purchased from Miles Laboratories, Elkhart, Ind. Samples used had the following lot numbers 2-4317, 6-4317, 11-10-317, 11-13-317, and 11-14-317. The manufacturer reported base ratios of 1:1 and sedimentation coefficients, corrected to water at 20°, ranging between 4.17 and 9.94. The $(dG)_n \cdot (dC)_n$ polymers were also a product of Miles Laboratories. Their control numbers were: 10-4318, 11-1-318, 11-12-318, 11-13-318, and 11-16-318. The guanosine content of this polymer, in different samples, varied over the range 48.5% guanosine to 68% guanosine. Reported sedimentation coefficients, corrected to water at 20°, varied between 10.0 and 11.8. The sedimentation coefficients indicate that the two previously mentioned samples were high molecular weight complexes. Both were prepared by the action of DNA polymerase on the appropriate mixture of nucleoside triphosphates.

Deoxyribonucleotides. dAMP and dGMP were products of the California Foundation for Biochemical Research, Los Angeles, Calif., lot numbers 150391 and 350131, respectively; dCMP was obtained from Sigma Chemical Co., St. Louis, Mo., lot no. 128-684, and dTMP was purchased from Schwarz BioResearch, Orangeburg, N. Y.; lot no. TMP-5903.

Physical Measurements. The samples were prepared and rotatory spectra recorded exactly as described in previous

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[1] See Green and Mahler (1970) for an explanation of abbreviations used in this paper.

TABLE I: Molar Extinction Coefficients (ϵ_{max}) of Polynucleotides and Mononucleotides (Aqueous Solution).

Material	λ_{max}[b]	ϵ_{max}[c]	Reference
$(dAdT)_n \cdot (dAdT)_n$	262	6.7	Inman and Baldwin (1964)
$(dG)_n \cdot (dC)_n$	253	7.4	Inman and Baldwin (1964)
Neutral $(dA)_n$	257	10.0	Ts'o *et al.* (1966)
$(dT)_n$	264	8.5	Ts'o *et al.* (1966)
DNA	260	6.6	Mahler *et al.* (1964)
dCMP	275	10.9	Voet *et al.* (1963)
dGMP	253	13.5	Voet *et al.* (1963)
dTMP	268	9.5	Voet *et al.* (1963)
dAMP	260	15.0	Voet *et al.* (1963)
DNA mixture[a]	261	11.0	Spirin *et al.* (1959)

[a] Mixture prepared in the following mole ratios: dGMP, 0.205; dCMP, 0.205; dAMP, 0.295; dTMP, 0.295. [b] Nanometers. [c] cm^2/mmole of P.

publications (Mahler *et al.*, 1968; Green and Mahler, 1970). As pointed out to us by one of the referees, with the instrument employed, absolute values of molar ellipticities and rotations as well as wavelength position of extrema, for all polymers are probably reliable (to a level of ±5%). Those for the monomers and certain single stranded polymers for which the absorbance, of the solutions used, was ≫ 1 at the absorption maximum may be in error by as much as 25% in value and 8 nm in wavelength.

Temperature Studies. For temperature studies, a temperature-jacketed cell, obtained from Optical Cell Co., Inc., Brentwood, Md., was employed. The path length used was 1.0 cm. The cells are furnished with stain-free end windows. For temperature control, a Forma-Temp Jr. Model 2095-2 was used. This instrument has a bath capacity of 7.5 gal. and a temperature range of −15 to +70°. The bath medium was a 1:1 mixture of water and glycol. This allowed temperatures as low as 5° to be attained without the formation of slush. Ice or slush formation would cause a loss in efficiency and give sluggish control. The connections between the spectropolarimeter and the temperature bath were made as short as possible to prevent heat gain, for temperatures below room temperature, or heat loss, for temperatures above room temperature. The system proved very efficient. For temperatures between 15 and 45°, there was no loss or gain of heat, measured to ±1°. For temperatures between 50 and 60°, there was a heat loss of approximately 1°. The procedure used for temperature studies was as follows: the cell was rinsed with the appropriate solvent and was then connected to the water bath. When the bath reached the desired starting temperature, the cell was filled with the sample solution and, at least, 0.5 hr was allowed for equilibration before spectra were recorded. The bath was then raised 5° and the same procedure followed, at 5° increments, until 60° was reached. Over the entire temperature range studied, the nucleic acid solution was left in the cell. No corrections for volume variations were made in any of the experiments described.

Results

DNA. We have previously compared and discussed optical rotatory dispersion data of selected DNAs in ethylene glycol and water solutions (Green and Mahler, 1968, 1970). Their absorbance and rotatory spectra in the former solvent were noticeably different from those of a component mixture of its mononucleotide components in either solvent. In addition, these DNAs in glycol melted out (measured either by a change of absorbance, circular dichroism or optical rotatory dispersion) in a highly cooperative fashion and with extensive hyperchromicity (30–40%). Our conclusion based on these observations, was that DNA underwent a major structural transition when its environment was changed from the aqueous to the nonaqueous one, and that its structure in the latter still possessed elements of order, which however, were not those inherent in the Watson-Crick-Wilkins (WCW) bihelical B form. The following studies were undertaken in order to characterize this unique structure further.

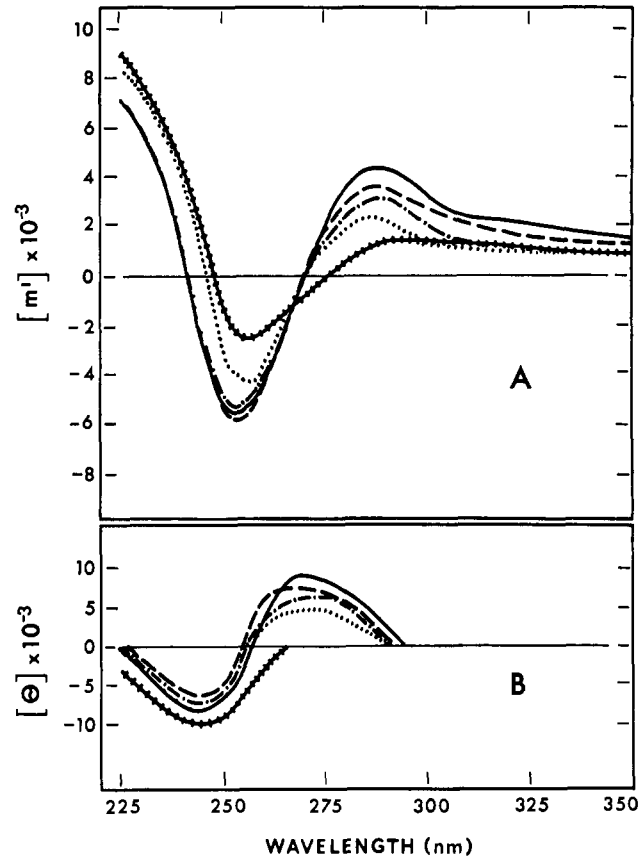

FIGURE 1: *E. coli* K-12 DNA in a series of water–glycol mixtures. (A) Optical rotatory dispersion and (B) circular dichroism. All spectra measured in 0.05 M KF–0.001 M EDTA (pH 5.8) at 27°. 100% HOH (——), 85% HOH (-----), 65% HOH (-·-·-), 35% HOH (·····), 5% HOH (+++++).

The experiments described in Figure 1 were performed, specifically, to see whether the changes observed when the solvent composition of a DNA solution was varied from pure water to pure glycol occurred gradually or abruptly over a very narrow range of solvent composition. The results appear straightforward. An inspection of the optical rotatory dispersion spectra shows the change in conformation to be broad and gradual in nature. Thus this structural change is noncooperative with respect to solvent composition and does not resemble melting of DNA in which changes are abrupt, and cooperative, and an all-or-none structural transition is observed over a very narrow temperature range (Table II). Specifically, the optical rotatory dispersion extremum at 287

TABLE II: Thermal Denaturation Parameters of DNA and Polynucleotides.[a]

Polynucleotide	T_m (°C)	$\sigma_{2/3}$ (°C)	h	h'
DNA (*E. coli*)	81.5	7.3	0.40	0.69
DNA (glycol)	39.3	4.2	0.34	0.49
Neutral $(dA)_n$	51.9	27.0	0.16	0.39
$(dA)_n \cdot (dT)_n$	55.5	12.5	0.23	
$(dA)_n \cdot (dT)_n$ (glycol)	22.0	9.2	0.24	
$(dAdT)_n \cdot (dAdT)_n$	55.1	3.4	0.41	1.24
$(dAdT)_n \cdot (dAdT)_n$ (glycol)	14.1	2.3	0.48	0.72
$(dG)_n \cdot (dC)_n$	88.5	8.0	0.17	0.67
$(dG)_n \cdot (dC)_n$ (glycol)	36.3	10.0	0.08	0.56

[a] All data measured in either aqueous or glycol medium (so indicated), 0.05 M KF–0.001 M EDTA (pH 5.5). T_m, $\sigma_{2/3}$, h, and h' are defined as follows (Green and Mahler, 1970). T_m equals the temperature at which $A_t/A_{8°} = 0.5\ A_{max}/A_{8°}$, where A_t, $A_{8°}$, and A_{max} are the absorbances (at the absorption maximum) at any temperature, at 8°, and its maximal value, respectively. $\sigma_{2/3}$ is defined as the temperature span for $(0.67\ A_{max}/A_{8°} - 0.33\ A_{max}/A_{8°})$. $h = (A_{max} - A_{8°}/A_{8°}$ for the polymer and $h' = [A((\text{mononucleotide}) - A_{8°})/A_{8°}]$. All thermal data recorded at λ_{max} except the last two samples which were recorded at 276 nm.

nm shows a progressive decrease in absolute rotation from its initial value of 4400 in pure water, to 3675, 3100, 2410, and 1200 when the water content in glycol–water mixtures is lowered to 85, 65, 35, and 5%, respectively. The crossover remains at 269 nm, except for the last instance where it is shifted to 276 nm. The optical rotatory dispersion minimum shows analogous decreases in rotation as a function of increasing glycol concentration.

Analogous behavior is seen in the circular dichroism spectra (Figure 1B) with all solutions, except the one in 5% water, still showing a gradually decreasing positive band. Unlike the positive extremum in the optical rotatory dispersion spectra the positive circular dichroism band not only decreases but also becomes broader as the glycol content is increased. It is easy to visualize this band as eventually becoming so broad as to gradually disappear at high glycol concentrations.[2] This broadening is seen, to a lesser extent, also with the negative band, but this band appears to first decrease and then increase in magnitude and only at the highest concentrations of glycol [95 and 100% (not shown, but identical with that in 95% glycol)] do changes become truly pronounced. Both sets of spectra show that the conformation characteristic of DNA in 100% glycol requires relatively high concentrations of this component. Even at 65% glycol–35% water, the similarities in conformation between DNA in this mixture and pure water are readily apparent, while it is also apparent that these conformations are both quite different from that of DNA in 95% glycol or above. Thus the alteration of conformation observed when water is replaced by glycol in solutions of DNA appear to occur in two steps: a principal one below ~90% glycol that is gradual and noncooperative and a second smaller, abrupt and probably cooperative one at about this concentration.

An interesting observation was made at 55% water–45% glycol, where apparently the result of some drastic change in base stacking is seen. At this particular glycol concentration, in contrast to the gradual changes in optical rotatory dispersion–circular dichroism, the absorbance increased from 0.38 to 0.48 (or 26%) and then again returned to the former value at high concentrations. This observation has been checked and rechecked for DNAs from four different organisms and found to be present in all samples. Such "intermediate" forms of DNA, stable only over an exceedingly narrow range, have previously been seen in viscosity measurements. This structure may correspond to an intermediate, perhaps partially unzippered form of DNA, a form through which the molecule must pass in the course of its transition from one stable conformation to the other.

The circular dichroism and optical rotatory dispersion parameters of the four relevant DNAs are summarized in Table III.

An interesting correlation emerges from the circular dichroism data: in water, both the position of the band maximum and its magnitude appear to depend on base composition: the former is blue shifted while the latter increases with increasing (G + C) content of the DNA. The blue-shift dependence is also observed for the position of the band minimum and is retained in glycol. As seen in the table the characteristic features of the optical rotatory dispersion and circular dichroism spectra of DNA dissolved in glycol described earlier are present in all instances and are independent of its base composition.

We observed that the different DNA samples appeared to require different amounts of ethylene glycol to produce $C_{1/2}$, one-half the maximal change in conformation when measured by optical rotatory dispersion, circular dichroism, or T_m (absorbance), and that these parameters were directly related to the guanine plus cytosine content of the particular DNA used. These results are summarized in Table IV. The compositional dependence was greatest for the $C_{1/2}$ (circular dichroism) —where 51% glycol was required for *Clostridium perfringens* compared to 75% glycol for *Escherichia coil* K-12—and least for $C_{1/2}$ (T_m), where these two samples required glycol concentrations differing by only 4%. These apparent discrepancies are not really surprising, since different parameters are sensitive to different aspects of conformational changes (Green and Mahler, 1970) and glycol may well exert its disruptive effect selectively on certain conformational features of the DNA molecule. Specifically, the changes in the—relatively local—dissymmetric environment to which circular dichroism (and optical rotatory dispersion) are responsive may depend a good deal more on base composition than do changes in melting point, particularly when the latter is measured by hyperchromicity—a variable that depends on much longer helical regions (Doty, 1962; Marmur and Doty, 1962; Marmur *et al.*, 1963; Felsenfeld and Miles, 1967). Glycol thus brings about an alteration in the local environment around the individual base pairs (and their immediate neighbors), but once this change has been effected the resultant conformation does not particularly depend on base composition in its stability to further disruption by heat. This interpretation is strengthened by two additional observations: except for the anomaly at 45% glycol the extinction coefficient

[2] After the completion of the experimental work incorporated in this paper, Nelson and Johnson (1970) communicated preliminary results concerning fine structure in the circular dichroism spectrum of DNA in glycol. We have therefore reinvestigated the circular dichroism spectrum of *E. coli* DNA in this solvent and can now confirm their results in all essentials.

TABLE III: Optical Rotatory Dispersion and Circular Dichroism Data for DNA Samples Used.

DNA	G + C (%)	Solvent	Optical Rotatory Dispersion: Positive Extremum λ (nm)	[m']	Optical Rotatory Dispersion: Negative Extremum λ (nm)	[m']	Circular Dichroism: Band Maximum λ (nm)	[θ]	Circular Dichroism: Band Minimum λ (nm)	[θ]
C. perfringens	27	H_2O	287	6290	255	−5040	274	4440	245	−8060
		Glycol	None		258	−3040	None		245	−7840
Salmon sperm	41	Glycol	None		261	−2130	None		245	−6745
Calf thymus	42	H_2O	287	4430	255	−4980	270	5775	242	−7400
		Glycol	None		259	−1800	None		240	−8325
E. coli K-12	50	H_2O	287	4660	255	−5740	270	8960	241	−8440
		Glycol	None		258	−2580	None		241	−6890

(or hypochromicity) of DNA at room temperature is quite insensitive to glycol concentration over the range between 0 and 100%, and its thermal transition in glycol solution is relatively insensitive to base composition (see also Table V and its discussion below).

Ts'o and Helmkamp (1961) have measured the effect of two other organic solvents on conformation, determined by optical rotatory dispersion of calf thymus DNA. The $C_{1/2}$ (optical rotatory dispersion) value for formamide was 70% while for dimethyl sulfoxide it was 58%, compared to our value for glycol of 65%. The change produced by dimethyl formamide is somewhat less gradual than that produced by glycol, while in dimethyl sulfoxide the change is abrupt and occurs over an extremely narrow range (±4%, v/v) of the organic component.

While the structure for DNA in glycol, at or below room temperature, remained unknown, we had already previously concluded that it contains considerable elements of order. One reason for this assertion is that DNA in glycol melts out, that is, can undergo a change in an optical parameter as a function of temperature. Previously we had used hyperchromicity (*i.e.*, absorbance); a further extension is illustrated in Figure 2 where we have plotted spectra of DNA as a function of temperature and the parameters measured were optical rotatory dispersion in part A and circular dichroism in part B.

The DNA spectrum, at 20 or 25°, is the same as that already reported in Figure 1. As the temperature is raised, the positive extremum, characteristic of the aqueous form, reappears and becomes progressively larger until 40° is reached. Between 40 and 60°, its magnitude remains constant and upon gradual, slow cooling, there is a slight increase in rotation and a noticeable shift to longer wavelengths. The same situation is seen for the negative extremum. There is a gradual increase in rotation between 20 and 35°, remaining constant from 35 to 60°. Upon cooling to 20°, there is a further increase in rotation, with all these changes centered about the same wavelength.

As shown in Figure 2B, the circular dichroism spectra show analogous behavior. At 20 and 25°, only the negative band is seen, centered at 241 nm. At 30°, the positive band reappears, increases in strength up till 40° and remains constant between 40 and 60°. Cooling the sample to 20° shifts the positive band center to longer wavelength by 8 nm, with a slight decrease in ellipticity. Simultaneous to this increase in rotational strength of the positive band with increasing temperature, a decrease is seen in the negative band until, at 40°, it is practically zero. There is no change in the wavelength maximum of the negative band.

Apparently then the optical rotatory dispersion spectrum of DNA, dissolved in glycol, and heated beyond its melting point, bears some superficial resemblance to that of the molecule in aqueous solution at room temperature. The circular dichroism spectrum, in particular its negative band, is, however, noticeably different: the latter is virtually absent for glycol solutions at higher temperatures while it is a prominent feature of unheated aqueous solutions and becomes reduced only upon heating and recooling (see Mahler *et al.*, 1968; Green and Mahler, 1970). This is sufficient evidence to permit the inference that the conformation of DNA after thermal denaturation in glycol bears a close resemblance to the one assumed in water after thermal denaturation and reequilibration at room temperature.

DNA in water and glycol exhibits analogous, though in the second case much weaker, dependence on base composition of several parameters that define the thermal transition. Specifically, as shown in Table V, T_m is directly dependent on guanine plus cytosine content. For every DNA, the T_m's measured by optical rotatory dispersion or circular dichroism

TABLE IV: Concentration of Glycol Required for One-Half-Maximal Change ($C_{1/2}$) as Determined from Circular Dichroism, Optical Rotatory Dispersion, and T_m.[a]

DNA	(G + C) Content (%)	$C_{1/2}$ (Circular Dichroism) (%)	$C_{1/2}$ (Optical Rotatory Dispersion) (%)	$C_{1/2}$ (T_m) (%)
C. perfringens	27	51	65	60
Salmon sperm	41	57	65	62
Calf thymus	42	57	65	63
E. coli K-12	50	75	76	64

[a] All data recorded in 0.05 M KF–0.001 M EDTA (pH 5.8) in water–glycol mixtures. The following parameters were used: for circular dichroism, the positive band center at 270 nm, for optical rotatory dispersion, the peak to trough magnitude of the first Cotton effect, both at room temperature; for T_m, the midpoint of the absorption increase at 260 nm.

TABLE V: Melting Transitions of Various DNA Samples.[a]

DNA	(G + C) Content (%)	Glycol					Water		
		T_m (Circular Dichroism) (°C)	T_m (Optical Rotatory Dispersion) (°C)	T_m (abs) (°C)	$\sigma_{2/3}$ (abs) (°C)	h (abs)	T_m (abs) (°C)	$\sigma_{2/3}$ (abs) (°C)	h (abs)
C. perfringens	27	30	31	33.9	3.0	0.39	71.5	4.0	0.36
Salmon sperm	41	33	34	37.0	4.7	0.31	77.5	7.1	0.39
Calf thymus	42	33	34	37.2	6.1	0.46	77.5	6.5	0.37
E. coli K-12	50	35	35	39.3	4.2	0.34	81.5	7.3	0.40

[a] Data recorded in 0.05 M KF–0.001 M EDTA (pH 5.8) in glycol, except the last three columns, which were run in aqueous solution. The following wavelengths were used: for circular dichroism, the positive band center at 270 nm; for optical rotatory dispersion, the peak at 287 nm; for absorption, the absorption maximum at 260 nm. The presence of noise did not allow T_m (circular dichroism) and T_m (optical rotatory dispersion) to be measured with as great an accuracy as the thermal-absorbance data.

TABLE VI: Optical Rotatory Dispersion Data.[a]

Material	First Extremum (nm)	$[m']_1$	First Crossover (nm)	Second Extremum (nm)	$[m']_2$	Second Crossover (nm)	$\pm([m']_1 - [m']_2)$
$(dA)_n$-neutral (water)	282	+2180	265	254	−2,360	248	4,540
$(dA)_n$-neutral (glycol)	257	−3540	243	227	+3,180		6,720
dAMP-neutral (water)	275	−1820	248	238	+540	227	2,360
dAMP-neutral (glycol)	273	−2420	240	235	+420	227	2,840
$(dT)_n$ (water)	288	+6300	275	261	−14,100	242	20,400
$(dT)_n$ (glycol)	286	+3390	277	260	−9,100	237	12,490
dTMP (water)	285	+1050	275	253	−4,420		5,470
dTMP (glycol)	286	+2950	275	255	−6,950		9,900
$(dA)_n \cdot (dT)_n$ (water)	291	+3660	280	255	−9,990	248	13,650
$(dA)_n \cdot (dT)_n$ (glycol)	290	+1330	282	261	−4,665	247	5,995
dAMP + dTMP (water)	287	+500	285	256	−3,550		4,050
dAMP + dTMP (glycol)	290	+750	287	273	−4,450	225	5,200
$(dAdT)_n \cdot (dAdT)_n$ (water)	283	+4000	263	255	−9,000	246	13,000
$(dAdT)_n \cdot (dAdT)_n$ (glycol)	282	+1500	275	261	−5,700	245	7,200
dAMP + dTMP (water)	287	+500	285	256	−3,550		4,050
dAMP + dTMP (glycol)	290	+750	287	273	−4,450	225	5,200
$(dG)_n \cdot (dC)_n$ (water)	307	+6600	260	248	−18,700	230	25,300
$(dG)_n \cdot (dC)_n$ (glycol)	301	+4550	266	250	−13,300	228	17,850
dGMP + dCMP (water)	290	+2750	276				
dGMP + dCMP (glycol)	293	−3500	280				

[a] Note: for $(dA)_n \cdot (dT)_n$ and $(dG)_n \cdot (dC)_n$, only the highest magnitude extrema are used.

are approximately equal while that measured by absorption is 3 or 4° higher. Our interpretation of this observation is again in terms of a disturbance of the local dissymmetric environment around individual base pairs before the complete disruption of the polymer itself. Thus, optical rotatory dispersion and circular dichroism measurements have shown themselves to be a delicate probe to detect changes prior to the onset of hyperchromicity.

A composite plot of the thermal transition midpoints of the four different DNAs in water or glycol as a function of their base composition is shown in Figure 3. The slope of T_m (abs) *vs.* (G + C) content is greater in water than in glycol, while the slopes for the three measurements $C_{1/2}$ (circular dichroism), $C_{1/2}$ (optical rotatory dispersion) and T_m (abs) in glycol are the same. The slope for aqueous solutions equals 0.47°/mole % (G + C), while for solutions of these DNAs in ethylene glycol the slope equals 0.25°/mole % (G + C), or approximately half that observed in water. These observations in conjunction with those just presented in Table IV indicate that optical parameters and thermal stability of DNA solutions

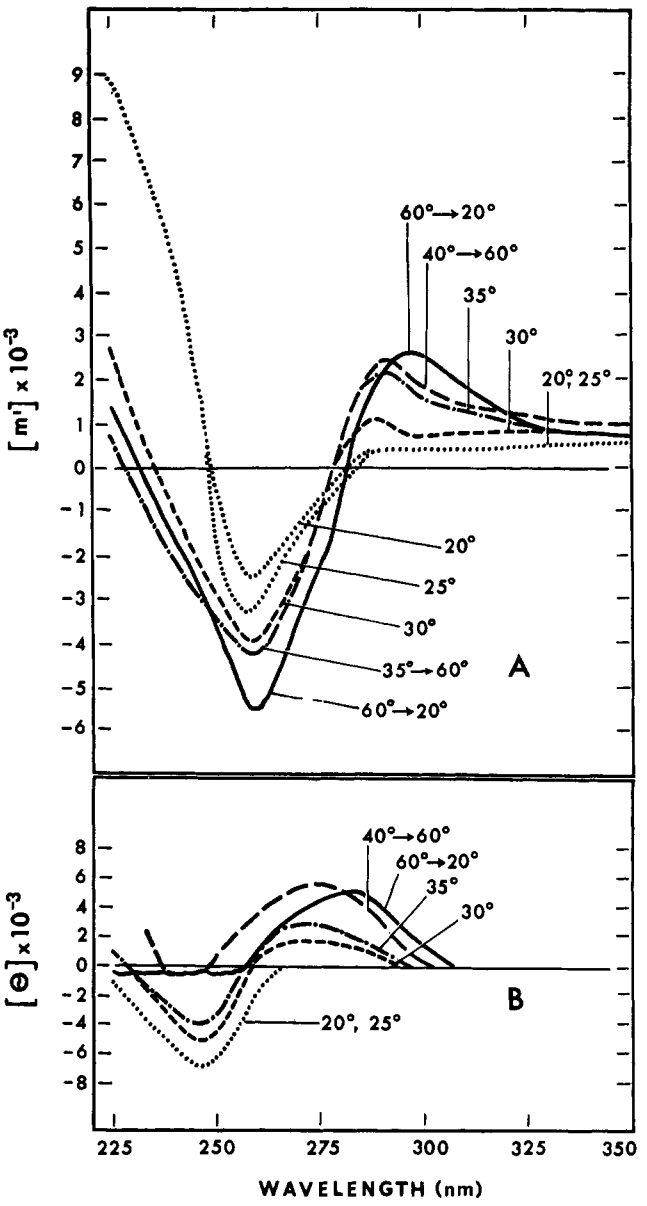

FIGURE 2: Optical rotatory dispersion and circular dichroism spectra of *E. coli* DNA plotted as a function of temperature. DNA was dissolved in 0.05 M KF–0.001 M EDTA–glycol, placed in a temperature-jacketed cell and the spectra recorded every five degrees on a slow scan. (A) Optical rotatory dispersion and (B) circular dichroism. Temperature at which scan was run is indicated on spectrum. 60 → 20° refers to the spectrum of DNA which was heated to 60° and then annealed to 20°. The spectrum, in this case, was recorded at 20°.

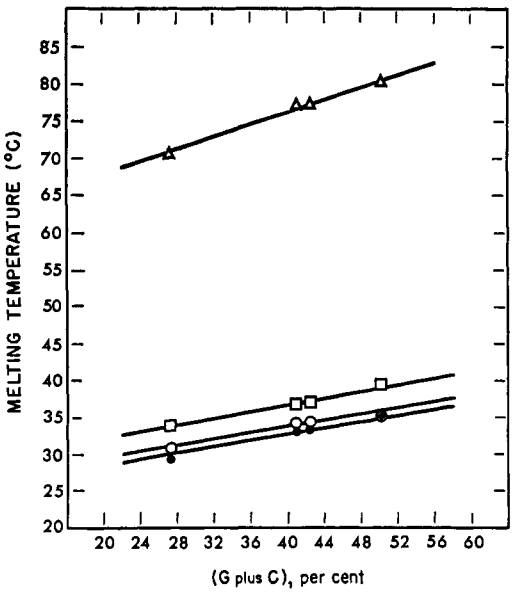

FIGURE 3: Melting transitions of DNAs as a function of guanine plus cytosine content. Conditions used are described in the legend of Figure 1. T_m circular dichroism (●●●●●), T_m optical rotatory dispersion (○○○○○), and T_m (abs) (□□□), all in glycol; T_m (abs) (△△△△△) aqueous solutions.

in water are much more sensitive to base composition than holds true in glycol. As a corollary then, base composition is not as important a determinant of DNA stability and conformation in glycol as it is in aqueous solution. An alternative explanation is that forces other than those depending principally on the presence of guanine and cytosine in a duplex are more important in determining helix stability in ethylene glycol than they are in water.

As in our previous study, we next sought to obtain comparisons between certain synthetic polymers (specifically here the polydeoxyribonucleotides) and DNA.

Polydeoxyriboadenylic acid $(dA)_n$. The appropriate spectra for $(dA)_n$ are shown in Figure 4. The properties of this polymer have been previously described (Riley *et al.*, 1966; Ts'o *et al.*, 1966; Vournakis *et al.*, 1967; Bush and Scheraga, 1969). Ts'o *et al.* (1966) have assigned it a single-stranded conformation at both pH 5.8 and 7.2 in contrast to the ribo analog, which at pH 5.8 is presented in a double-helical form. The location of the absorption maximum at 257 nm provides additional evidence for a single-stranded conformation since the double-stranded form shows an absorption maximum above 260 nm (Ts'o *et al.*, 1966).

The optical rotatory dispersion spectrum reported here is in good agreement with one previously reported (Ts'o *et al.*, 1966). In glycol, the spectrum qualitatively resembles that for the monomers with the positive Cotton effect of the aqueous polymer replaced by a negative one. The wavelength of the first extremum in glycol occurs within 3 nm of the trough of the aqueous solution with an apparent small increase in the value of $[m']$. The second long-wavelength peak in the aqueous solution (Ts'o reports a value of $[m']$ of 27,000 at a wavelength of 240 nm) has been sharply reduced to a value of 3180 with a blue shift to 227 nm. The monomers exhibit the negative Cotton effect characteristic of purine β-nucleotides with both solutions exhibiting nearly identical magnitudes and wavelengths of the extrema. Their values are in the same range as ones previously published (Yang *et al.*, 1966; Adler *et al.*, 1969; Bush and Sheraga, 1969); differences are probably due to instrument error.

More precise interpretations can be derived from the circular dichroism spectra, which, for the polymer in aqueous solution, consist of a negative and a positive band. The latter, centered at 273 nm, with a $[\theta]$ of 3300 appears to be the result of more than one transition. It is symmetrical, but very broad in nature, broader in fact and lower in magnitude than that reported recently for the dimer (Cantor *et al.*, 1970). The negative band, while of greater magnitude than the positive one appears to be much narrower. In glycol, the positive band disappears while the negative band remains, albeit with a reduced value of $[\theta]$ (from 12,800 to 7100). Its band center is at 249 nm compared to an absorption maximum at 259 nm. Thus this circular dichroism band cannot simply be taken as a

TABLE VII: Circular Dichroism Data.[a]

Material	Band Max (nm)	$[\theta]_{max}$	First Crossover (nm)	Band Min (nm)	$[\theta]_{min}$	Second Crossover (nm)
$(dA)_n$-neutral (water)	273	+3,300	257	248	−12,800	239
$(dA)_n$-neutral (glycol)				249	−7,100	233
dAMP-neutral (water)				260	−3,200	233
dAMP-neutral (glycol)				260	−2,600	241
$(dT)_n$ (water)	275	+15,590	262	250	−12,000	
$(dT)_n$ (glycol)	273	+10,550	260	245	−8,620	
dTMP (water)	272	+5,240	255	235	−4,310	
dTMP (glycol)	271	+6,450	256	237	−6,760	
$(dA)_n \cdot (dT)_n$ (water)	263	+4,500	259	250	−23,500	236
$(dA)_n \cdot (dT)_n$ (glycol)	276	+2,500	265	246	−6,500	
dAMP + dTMP (water)	275	+1,000	270	243	−3,000	
dAMP + dTMP (glycol)	277	+750	271	250	−3,100	
$(dAdT)_n \cdot (dAdT)_n$ (water)	264	+4,750	257	248	−8,750	230
$(dAdT)_n \cdot (dAdT)_n$ (glycol)	272	+2,800	263	250	−3,375	
dAMP + dTMP (water)	275	+1,000	270	243	−3,000	
dAMP + dTMP (glycol)	277	+750	271	250	−3,100	
$(dG)_n \cdot (dC)_n$ (water)	257	+16,265	247	240	−8,200	
$(dG)_n \cdot (dC)_n$ (glycol)	262	+13,650	248	238	−6,475	
dGMP + dCMP (water)	265	+5,325	250			
dGMP + dCMP (glycol)	265	+6,650	240			

[a] Note: for $(dA)_n \cdot (dT)_n$ and $(dG)_n \cdot (dC)_n$, the values shown are those of the most intense extrema.

"nonconservative" product generated by the transition responsible for the absorption band, but must be more complex in origin, and is perhaps in part due to a "conservative" transition around 230 nm, the crossover wavelength (see also Bush and Sheraga, 1969). The spectra, particularly with regard to band position, of the monomers are quite similar in the two solvents, with dAMP in water showing a slightly greater rotational strength. The band position, shape, and increased rotational strength of the polymer in glycol relative to its monomers in this solvent may be taken as evidence for the retention of some degree of stacking with a helical sense. This conclusion is further substantiated by an inspection of the absorption spectra. In glycol, the polymer becomes only slightly hyperchromic, *i.e.*, an increase in ϵ_{max} from 10,050 to 10,690 with corresponding values for the monomers being 14,090 and 14,350. It is thus apparent that those base–base interactions responsible for the hypochromism of the polymer remain relatively unaffected by the change in medium.

TABLE VIII: Absorption Data.

Material	λ_{max} (nm)	ϵ_{max} (cm^2/mmole of P)
$(dA)_n$-neutral (water)	257	10,050
$(dA)_n$-neutral (glycol)	259	10,690
dAMP-neutral (water)	260	14,090
dAMP-neutral (glycol)	262	14,350
$(dT)_n$ (water)	265	8,640
$(dT)_n$ (glycol)	267	8,460
dTMP (water)	267	10,080
dTMP (glycol)	266	12,000
$(dAdT)_n \cdot (dAdT)_n$ (water)	263	6,750
$(dAdT)_n \cdot (dAdT)_n$ (glycol)	264	7,350
dAMP + dTMP (water)	260	14,700
dAMP + dTMP (glycol)	265	12,600
$(dG)_n \cdot (dC)_n$ (water)	260	7,670
$(dG)_n \cdot (dC)_n$ (glycol)	255	7,300
dGMP + dCMP (water)	256	12,650
dGMP + dCMP (glycol)	258	11,680

Figure 4 and Tables VII and VIII show that while the wavelengths of the center of the negative circular dichroism band and the absorption maximum are within 2 nm of one another for the monomer in both solvents, this is not true for the polymer in either solvent. Previous observations (see Green and Mahler, 1970) suggested that whenever the positions of a circular dichroic and absorption band coincide in a polymer, its individual bases appear to be oriented at random, whereas when the two-band maxima differ in the same solvent this is indicative of the retention of some order. An explanation of this observation is provided by Tinoco's theories of optical activity which assign a variable conservative component (with a *crossover* at the absorption maximum) to the spectra of all *n*-mers ($n \geq 2$) in which the rotational angle between adjacent bases is not equal to 0 or 180° (Tinoco and Woody, 1963; Tinoco, 1964; see also Cantor *et al.*, 1970).

Thus, there is pronounced *similarity* in the structure of DNA and $(dA)_n$ in glycol at room temperature as indicated by their very close correspondence in two of the three optical criteria, namely, (a) in glycolic solution the positive circular dichroism band characteristic of the aqueous solution is virtually eliminated, while the negative band is retained; co-

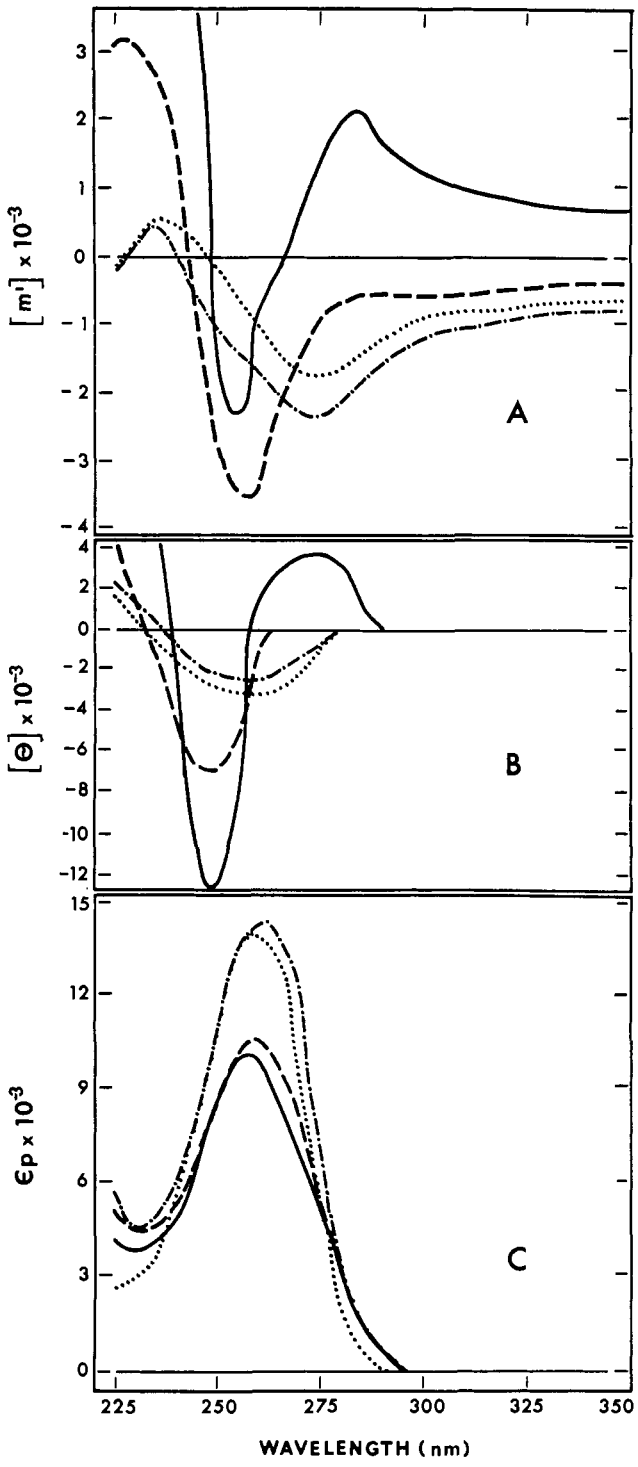

FIGURE 4: Optical spectra for neutral $(dA)_n$ and dAMP in 0.05 M KF–0.001 M EDTA (pH 5.8). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. $(dA)_n$ in water (——), $(dA)_n$ in glycol (-----), dAMP in water (· · · · ·), dAMP in glycol (-·-·-). Concentration for optical rotatory dispersion and circular dichroism was 3.7×10^{-4} M polymer phosphate, while 3.7×10^{-5} M polymer phosphate was used for the absorption spectra. Rotatory and absorption spectra were recorded at 27 ± 1° in a 1-cm cell.

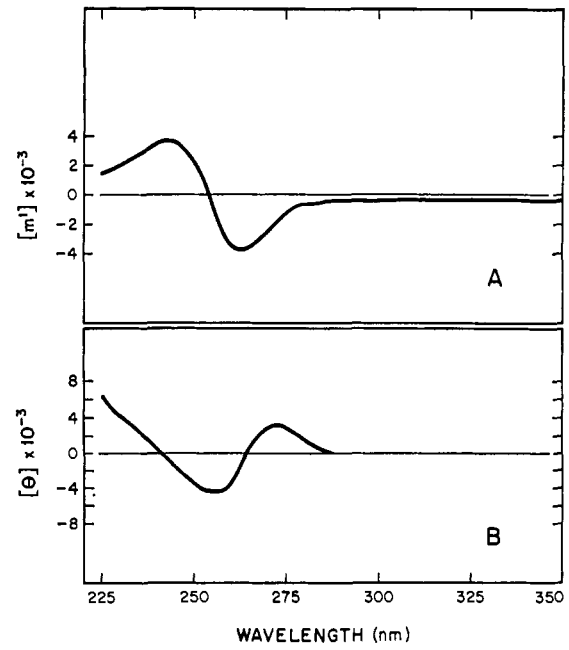

FIGURE 5: Optical spectra for neutral $(dA)_n$ in 0.05 M KF–0.001 M EDTA in glycol medium. (A) Optical rotatory dispersion and (B) circular dichroism. Concentration for optical rotary dispersion and circular dichroism was 4.02×10^{-4} M polymer phosphate. Spectra were recorded at 6 ± 1° in a 1-cm cell.

incident with (b) only a slight increase in extinction coefficient of the polymer solution is seen in glycol as compared to water. The behavior of the two polymers differs when a comparison is made between their glycolic solution and that of their constituent monomers in either water or glycol. While the circular dichroism and optical rotatory dispersion spectra of the mixture of component mononucleotides in no way resembles that of DNA in glycol, for $(dA)_n$ the qualitative resemblance, in the sign at least, is strong.

A permissible interpretation of these data is that although the structures of DNA and $(dA)_n$ [but not $(rA)_n$!] in glycol exhibit certain similarities, probably referrable to interactions between adenine bases on the same strand, the structure and spectra of DNA in addition depend on interactions with one or more of the other bases. These probably do not involve interstrand (horizontal) interactions with thymine, to any great extent, since the $(dA)_n \cdot (dT)_n$ duplex exhibits a qualitatively different behavior (see below).

When the spectra of $(dA)_n$, were recorded under the conditions just described, but at a lower temperature, *i.e.*, 6 ± 1°, the curves in Figure 5 were obtained. The sample here was prepared by directly dissolving $(dA)_n$ in glycol, keeping all materials at 4–6°, and then placing the sample solution in the temperature-jacketed cell at 6 ± 1°. The optical rotatory dispersion spectra, for $(dA)_n$ in glycol at 6° and at 27° are quite similar (compare Figure 5 and Figure 4A). At the lower temperatures, the extrema are shifted to longer wavelengths, *i.e.*, from 257 to 262 nm for the trough and from 227 to 242 nm for the peak. The absolute magnitudes at the two temperatures are, however, comparable.

A noticeable difference in conformation of the polymer, at the two temperatures, does however, become apparent from the circular dichroism spectra. In Figure 5B one can detect not only the presence of a positive band with a $[\theta]$ and λ_{max} very similar to those of the aqueous form of $(dA)_n$ (Figure 4B), but in addition an apparently conservative negative band, broader than that of the aqueous form and with its maximum approximately one-third in magnitude and shifted 7 nm to longer wavelengths. Thus, even this low-temperature form of $(dA)_n$ in glycol, while exhibiting some similarity in geometry to the aqueous form, cannot be identical with it. Further evidence for

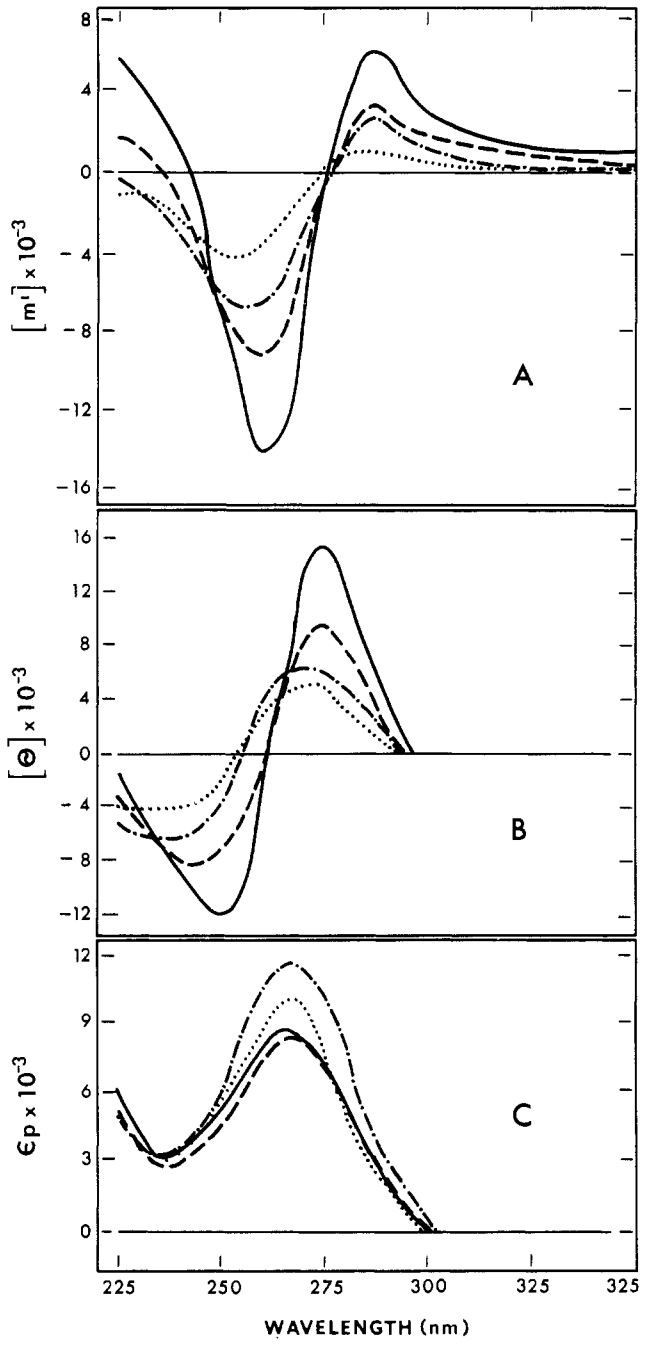

FIGURE 6: Optical spectra for $(dT)_n$ and dTMP in 0.05 M KF–0.001 M EDTA (pH 5.8). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. $(dT)_n$ in water (——), $(dT)_n$ in glycol (-----), dAMP in water (· · · · ·), dAMP in glycol (-·-·-). Concentration for optical rotatory dispersion and circular dichroism was 2.7×10^{-4} M polymer phosphate, while 2.7×10^{-5} M polymer phosphate was used for the absorption spectra. Rotatory and absorption spectra were recorded at 27 ± 1° in a 1-cm cell.

this assertion is derived from the thermal absorbance profiles (to be discussed later) where no distinct thermal transition is seen in glycolic solutions not even at low temperatures while a broad transition is observed in aqueous solution.

Polydeoxyribothymidylic Acid, $(dT)_n \cdot (dT)_n$ was chosen as a second example of a synthetic single-stranded deoxyribopolymer. Some of its properties have been previously reported (Riley *et al.*, 1966; Ts'o *et al.*, 1966). Its spectra are shown in Figure 6. These data agree very well, both qualitatively and quantitatively, with those previously published (Ts'o *et al.*, 1966).

Previous data have been interpreted as showing that $(dT)_n$ exhibits very little stacking interaction so that its secondary structure is probably close to that of a random coil, even at 1°. The presence of a 2′-OH can stabilize this type of polymer since the ribo analog exhibits a higher T_m (Ts'o *et al.*, 1966). An examination of Figure 6 shows, however, that some stacking or incipient helical order must be present in the polymer in aqueous solution since it exhibits a significantly smaller extinction coefficient and greater rotation and ellipticity than either the polymer in glycol or the mononucleotides in either solvent—or even the dinucleotide in water (Cantor *et al.*, 1970). In glycol, the optical rotatory dispersion spectrum of $(dT)_n$ shows a decrease (relative to water) in magnitude of the first Cotton effect from 20,400 to 12,490 or 33%. This is considerably less than the decrease in going to the monomer in glycol (51%) or in water (74%). Similar observations characterize the circular dichroism spectra and both sets of data lead to the same conclusion, namely, that the dissymmetry is reduced, but not eliminated, in glycolic solution, and that the polymer, in either solvent exhibits a greater degree of dissymmetry (or short range order) than is found in the monomers or dimers in aqueous solution.

Polydeoxyriboadenylic·Polydeoxyribothmidylic Duplex, $(dA)_n \cdot (dT)_n$. The rotatory spectra for this duplex obtained by us and shown in Figure 7, agree quite well with those previously published (Chamberlin, 1965; Ts'o *et al.*, 1966; Bernardi and Timasheff, 1970). X-Ray studies on $(dA)_n \cdot (dT)_n$ fibers indicate that the structure does not resemble the B form of DNA (Davies and Baldwin, 1963; Langridge, 1966), perhaps due in part to the different diameters of the two strands; apparently this difference is sufficient to account for the observed nonconservative rotatory spectra. The optical rotatory dispersion and circular dichroism spectra for the aqueous solution of the $(dA)_n \cdot (dT)_n$ polymer are quite complex and are characterized by three peaks and two troughs in the wavelength range 225–350 nm. The three long-wavelength extrema are of low magnitude exhibiting values of 3660 (at 291 nm), 2950 (at 273 nm), and 2000 (at 265 nm). The short-wavelength peak at 237 nm has a $[m']$ value of −17,340. In glycol, at room temperature, the curve becomes simplified and resembles that seen for the monomers (see Figure 7A). The five extrema of the aqueous solution are replaced by three broad ones with a peak to trough magnitude for the first positive Cotton effect of 5995. This compares to 4050 and 5200 for the corresponding mononucleotide mixtures in water and glycol, respectively, and we conclude that most of the dissymmetry has been lost.

The same conclusions can be drawn from the circular dichroism spectra (Figure 7B). That of the aqueous solution is complex, exhibiting four bands and the beginnings of a fifth one. In glycol, this pattern is reduced to two bands, a positive one at longer wavelength and a negative one at shorter wavelength. The mononucleotide mixture exhibits the same two bands, albeit of even more reduced magnitude. These observations suggest that the conformation of the polymer in glycol at room temperature is similar to that of a solution of the monomers but that some structural constraints are still operative at this temperature. These however are not as great as those maintained in either of the mixed dinucleotides dAdT or dTdA (Cantor *et al.*, 1970).

Since the $(dA)_n \cdot (dT)_n$ duplex in glycol can undergo a thermal transition (see later discussion) we also examined the spectra in glycol below 20°, in order to characterize the low-temperature form and compare it to DNA. These spectra, at

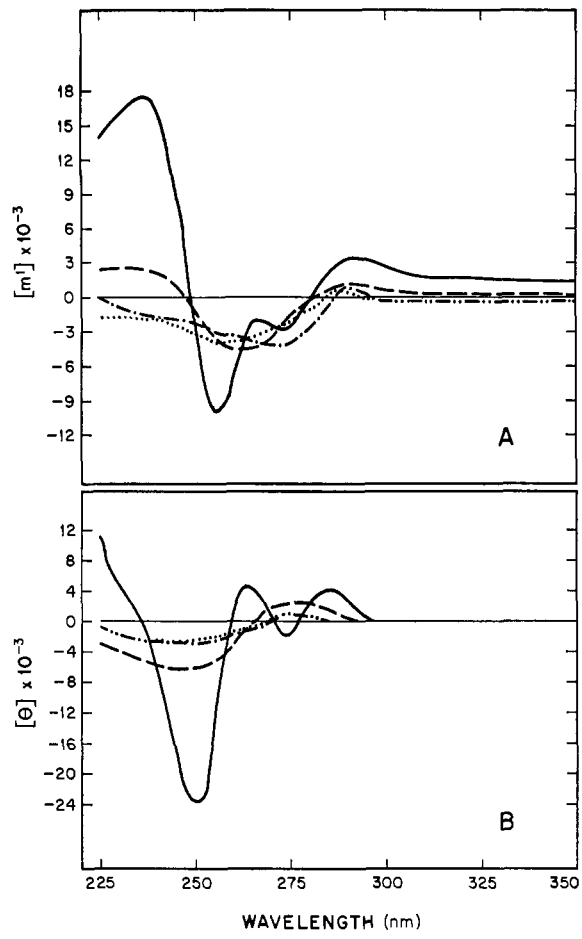

FIGURE 7: Optical spectra of $(dA)_n \cdot (dT)_n$ and a 1:1 mixture of dAMP and dTMP in 0.05 M KF–0.001 M EDTA (pH 5.8). (A) Optical rotatory dispersion and (B) circular dichroism. $(dA)_n \cdot (dT)_n$ in water (——), $(dA)_n \cdot (dT)_n$ in glycol (-----), dAMP plus dTMP in water (· · · · ·), dAMP plus dTMP in glycol (-·-·-). Concentration for optical rotatory dispersion and circular dichroism spectra was 1.3×10^{-4} M polymer phosphate. Spectra were recorded at $27 \pm 1°$ in a 1-cm cell.

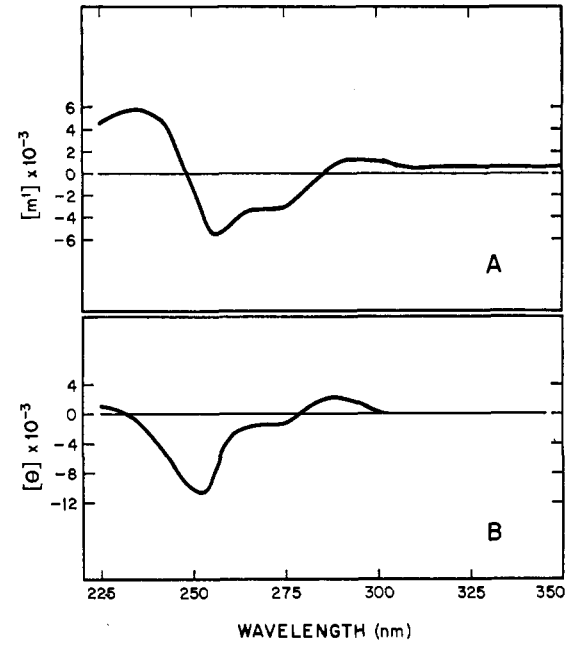

FIGURE 8: Optical spectra of $(dA)_n \cdot (dT)_n$ in glycol, 0.05 M KF–0.001 M EDTA (pH 5.8). (A) Optical rotatory dispersion and (B) circular dichroism. Concentrations for optical rotatory dispersion and circular dichroism was 1.3×10^{-4} M polymer phosphate. Spectra were recorded at $7 \pm 1°$ in a 1-cm cell.

$7 \pm 1°$, are presented in Figure 8. They appear to be those of a structure intermediate between that of the polymer in water and the one in glycol at or above room temperature. All the extrema in Figure 8A are reduced in magnitude compared to Figure 7A while the ones at 273 and 265 nm have become merged into one broad shoulder. Similar effects are seen in Figure 8B where the negative and positive bands at 273 and 262 nm in aqueous solution (see Figure 7B) have become reduced to a low-intensity plateau. When this solution is warmed to room temperature, the plateau disappears and the spectrum is now characterized only by the two broad bands similar to those of the mononucleotide mixture.

Duplex Copolymer of Polydeoxyriboadenylic and Thymidylic Acid, $(dAdT)_n \cdot (dAdT)_n$. The representative spectra for this duplex are shown in Figure 9. The solid polymer is known to consist of two chains forming a double helix in a B conformation similar to that of DNA, as determined from X-ray diffraction measurements on their lithium salts (Davies and Baldwin, 1963). In this heteropolymer, thymine and adenine alternate in complete regularity which allows for perfect base pairing along the entire length of the chains (Inman and Baldwin, 1962; Chamberlin *et al.*, 1963; Davies and Baldwin, 1963; Ts'o *et al.*, 1966). This also facilitates complete renaturation upon cooling of a heated solution of the duplex (Marmur and Doty, 1959). Synthetically prepared $(dAdT)_n \cdot (dAdT)_n$ has been shown to have branches and hairpin-like structures brought about through intrachain base pairing (Inman and Baldwin, 1962; Inman *et al.*, 1965). Figure 9A shows the optical rotatory dispersion spectrum of the polymer in aqueous solution. This spectrum agrees very well with that obtained by Ts'o *et al.* (1966), both with regard to wavelengths and absolute values for the extrema. Upon transfer to glycol at room temperature, there is observed a sharp decrease in both the peak (4000–1500) and trough (9000–5700) with a corresponding shift in the crossover point to longer wavelength (263–275 nm). In spite of these sharp reductions in magnitude, the positive Cotton effect is retained and constitutes a characteristic part of the optical rotatory dispersion spectrum. Thus in glycol solution under these conditions DNA and the $(dAdT)_n \cdot (dAdT)_n$ duplex must differ in important conformational parameters. The spectra for the 1:1 mixture of mononucleotides in water or glycol are similar, with a peak to trough magnitude in water of 4050 increased to 5200 in glycol. Both values are, however, lower than the value seen for the polymer in glycol, which is 7200, an indication of the retention of some form of dissymmetry. An inspection of the circular dichroism and absorption curves provides further substantiation. The circular dichroism spectrum of $(dAdT)_n \cdot (dAdT)_n$ in water is roughly conservative with a crossover at 257 nm. The positive band however, is skewed similar to that of DNA (Figure 1) and appears to be composed of two overlapping unequal bands with the smaller one occurring at longer wavelengths. There is no indication of this complexity in the circular dichroism spectra of the two-component dinucleotides d(ApT) and d(TpAp) published by Cantor *et al.* (1970) and its magnitude in both these instances is *larger* than that found in the polymer. The optical rotatory dispersion spectrum is also relatively simple in this region, although an inspection of the absorption curve shows that in the region of 290–300 nm the polymer in

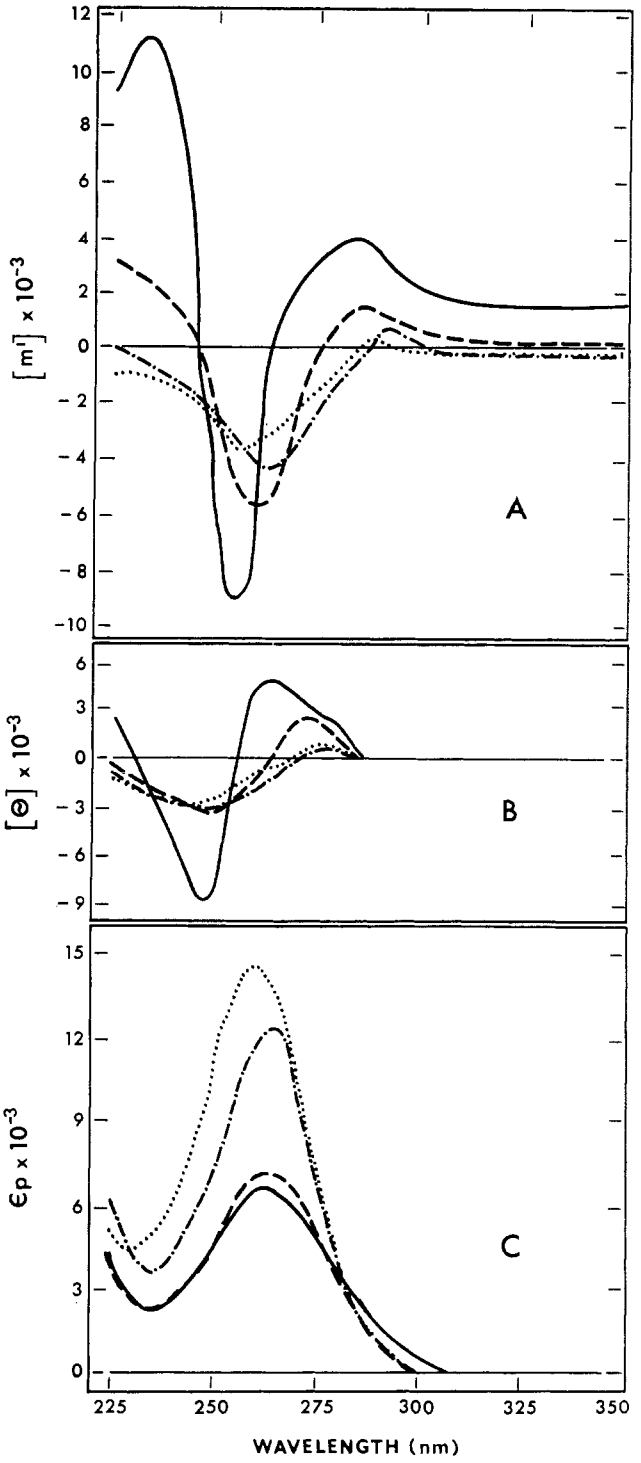

FIGURE 9: Optical spectra of $(dAdT)_n \cdot (dAdT)_n$ and a 1:1 mixture of dAMP and dTMP in 0.05 M KF–0.001 M EDTA (pH 5.5). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. $(dAdT)_n \cdot (dAdT)_n$ in water (——), $(dAdT)_n \cdot (dAdT)_n$ in glycol (-----), dAMP plus dTMP in water (· · · · ·), dAMP plus dTMP in glycol (-·-·-). Concentration for optical rotatory dispersion and circular dichroism spectra was 2.1×10^{-4} M polymer phosphate, while 4.2×10^{-5} M polymer phosphate was used for the absorption curves. Rotatory and absorption spectra were recorded at 27 ± 1° in a 1-cm cell.

aqueous solution is *hyperchromic* relative to the monomers in either solvent or to the polymer in glycol. This set of observations is characteristic of a n → π* transition as has been discussed previously (Green and Mahler, 1970).

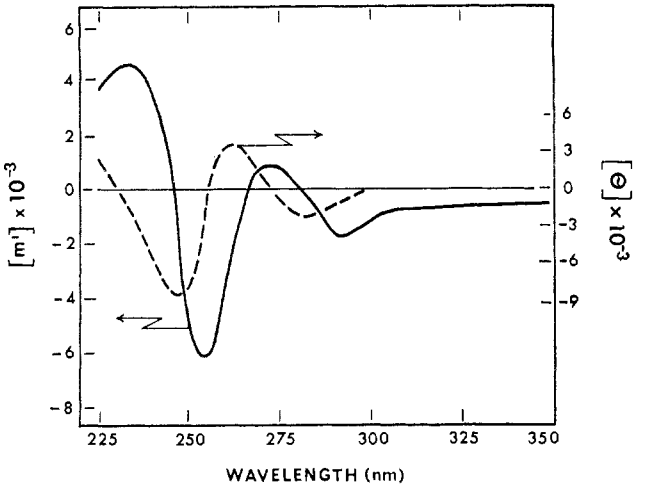

FIGURE 10: Optical spectra of $(dAdT)_n \cdot (dAdT)_n$ in glycol, 0.05 M KF–0.001 M EDTA (pH 5.5). Optical rotatory dispersion (——) and circular dichroism (-----). Concentration for optical rotatory dispersion and circular dichroism was 2.4×10^{-4} M polymer phosphate. Spectra were recorded at 8 ± 1° in a 1-cm cell.

Interesting features emerge from a comparison of the circular dichroism spectra of the mononucleotides (in water or glycol) with that of the room temperature form of the polymer in glycol. All three samples exhibit a positive and a negative band. While the three negative bands are similar in magnitude, *i.e.*, $[\theta]$ values of 3000, 3100, and 3375, there is a noticeable difference in the size of the residual positive band. The polymer in glycol exhibits a value of $[\theta]$ of 2800 which is approximately three times the value for the monomers in water and four times that of the monomers in glycol (see Table VII). Coincident with this retention of rotational strength the absorption spectrum indicates only a slight hyperchromicity for the polymer in going from water to glycol solution, with an increase in ϵ_p from 6750 to 7350. In contrast, the monomers in aqueous and glycol solution show ϵ_p values of 14,700 and 12,600, respectively. Furthermore the wavelength of maximum absorption for the polymer in glycol coincides with that of the crossover in the circular dichroism spectrum. The two bands of the latter are approximately equal, and hence conservative, and in conjunction with the other evidence indicative of the retention of some helical order; however, the structure responsible must be both qualitatively and quantitatively different from the one present in aqueous solution, and probably (see below) does not correspond to a simple double helix.

Since the polymer in glycol undergoes a thermal transition with a T_m at 14.1° (Table II) and the spectra described in Figure 9 were recorded at 27°, the optical data presented there are those of a polymer in which the native duplex structure has been altered. It was thus deemed important to measure the relevant parameters below the melting point. Figure 10 shows the results for $(dAdT)_n \cdot (dAdT)_n$, under conditions of pH and ionic strength identical with those for Figure 9, but at 8 ± 1°. For optical rotatory dispersion, in the wavelength range between 225 and 300 nm, two peaks and two troughs, all of low magnitude, are evident at 8°, but while the three short-wavelength extrema are evident also at 27°, the trough at ≅290 nm is present only at the lower temperature. Since we have tentatively assigned the transition at the longest wavelength to an n → π* excitation (Green and Mahler, 1970), we conclude that it is the geometry producing this transition that is principally affected when the polymer is heated in glycol. The circu-

lar dichroism spectra further substantiate this conclusion. In the wavelength range where one saw one band composed of two overlapping transitions in aqueous solution, we observe in glycol at the low temperature one positive and one negative band, with the negative one occurring at longer wavelengths and centered at 278 nm. Interesting also are the pronounced differences between the two dA and dT containing duplexes in glycol at low temperature (Figure 8 *vs.* Figure 10) retaining —in a modified form—the differences between their aqueous counterparts.

One apparent discrepancy in the above data concerns the fact that, on the one hand, when the polymer is dissolved either in water or glycol at room temperature, the absorption spectra are almost identical and hypochromic, while on the other, the polymer dissolved in glycol at low temperature melts out at 14.1° with a hyperchromicity of 0.48. Perhaps the structure assumed by the polymer dissolved in glycol at 8° and then brought to room temperature is not identical with that of the molecule dissolved in this solvent directly at that temperature. Since $(dAdT)_n \cdot (dAdT)_n$ is a polymer containing only two bases, it is possible to conceive of structures which are readily convertible into each other simply by the switching of intra to interstrand base pairs and *vice versa*. Similar observations, interpreted in an analogous manner, have previously been reported by Ts'o *et al.* (1966), on thermally induced optical rotatory dispersion changes with this polymer in aqueous solution. Further work will have to be done on the properties of the low- and room-temperature forms before this point can be settled.

In any event the lack of resemblance of the conformation of this polymer in glycol—even at low temperatures—to that of DNA in the same solvent is unexpected, considering that both lack the 2'-OH, their fiber X-ray diffraction patterns are similar and are generated by similar anti-parallel double helices. Perhaps the difference is related to the fact already alluded to that, especially in solution, $(dAdT)_n \cdot (dAdT)_n$ can form hairpin-like structures by means of intrachain base pairing (Inman and Baldwin, 1962; Inman *et al.*, 1965; Bernardi and Timasheff, 1970).

Polydeoxyguanylic·Polydeoxycytidylic Duplex, $(dG)_n \cdot (dC)_n$. The optical data for this complex are presented in Figures 11 and 12. Although these particular ones were all recorded at pH 5.5, those at pH 7.0 were identical. This behavior is different from that of the corresponding ribopolymer duplex which was shown to dissociate at the acidic pH (Green and Mahler, 1970). Enzymatically synthesized $(dG)_n \cdot (dC)_n$ consists of two homopolymer strands where less than 0.5% of the guanosine is covalently linked to cytidine (Inman and Baldwin, 1964). Base ratios for this polymer, however, have been shown to deviate from unity, and the guanosine content in the four samples used in the following experiments ranged from 48.5 to 68%. Results with a polymer containing 63% guanosine are shown in Figure 11, while the results of Figure 12 are representative of the other three polymers. Samejima and Yang (1965) reported the first optical rotatory dispersion spectrum of $(dG)_n \cdot (dC)_n$. Our spectra are similar, so far as wavelength positions of the extrema are concerned, although there are differences in their magnitude. For example, the magnitude of the first peak in our spectrum is greater than the second one, while the reverse has been reported by Samejima and Yang. These differences can probably be related to the methods of preparation (which could determine the amount or extent of branching) and possibly also the guanosine content of the polymer (compare Figures 11 and 12).

A most interesting observation made by the previously

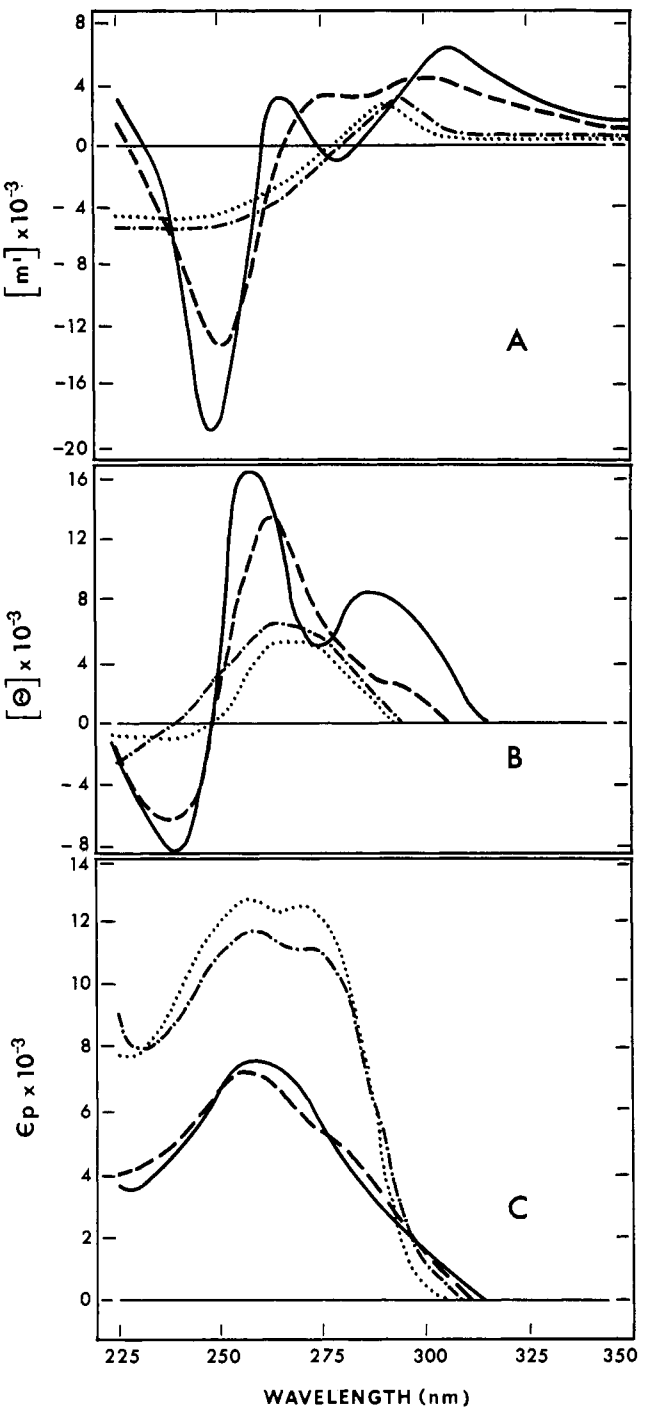

FIGURE 11: Optical spectra of $(dG)_n \cdot (dC)_n$, where percentage of dG is 63%, and mononucleotide components, in 0.05 M KF–0.001 M EDTA (pH 5.5). (A) Optical rotatory dispersion, (B) circular dichroism, and (C) absorption. $(dG)_n \cdot (dC)_n$ in water (——), $(dG)_n \cdot (dC)_n$ in glycol (-----), dCMP plus dGMP in water (· · · · ·), dCMP plus dGMP in glycol (-·-·-). Concentration for optical rotatory dispersion and circular dichroism spectra was 2.36×10^{-4} M polymer phosphate, while 4.7×10^{-6} M polymer phosphate was used for the absorption curves. Spectra were recorded at 27 ± 1° in a 1-cm cell.

mentioned authors was that with an increase in temperature, the positive Cotton effect was changed into a negative one, producing a spectrum very nearly identical with that shown in Figure 12. This leads to the conclusion that $(dG)_n \cdot (dC)_n$ can exist in, at least, two different conformations depending on base composition and temperature. The difference between

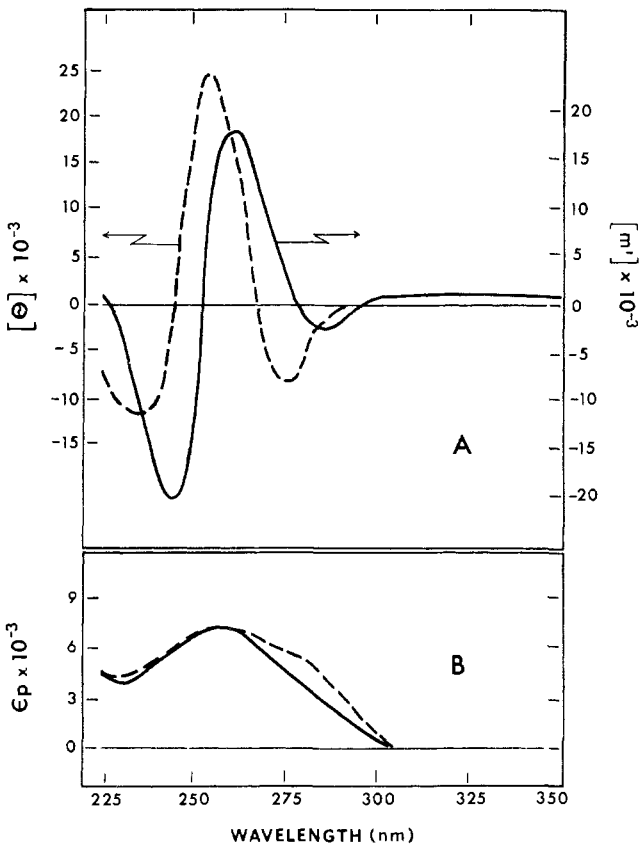

FIGURE 12: Optical spectra of $(dG)_n \cdot (dC)_n$, where percentage of dG is 58%, in 0.05 M KF–0.001 M EDTA (pH 5.5). (A) Optical rotatory dispersion (——) and circular dichroism (-----) in aqueous medium. (B) Absorption spectra. Water (——) and glycol (-----). Concentration for optical rotatory dispersion and circular dichroism spectra was 1.7×10^{-4} M polymer phosphate, while 2.4×10^{-5} M polymer phosphate was used for the absorption curves. Spectra were recorded at 27 ± 1° in a 1-cm cell.

these two forms may well be due to different forms and extent of branches or hairpin-like structures in the two strands [*cf.* the preceding section concerning analogous behavior of $(dAdT)_n$].

When the polymer producing the spectra recorded in Figure 11 is placed in glycol, a high degree of order appears to be retained, whether optical rotatory dispersion, circular dichroism, or absorption spectra is used as criteria. In the optical rotatory dispersion, the magnitude of the long-wavelength peak is reduced to 70% of its value in aqueous solution while the trough is decreased to 60%. The monomers show very similar spectra in both solvents.

Further evidence is provided by the circular dichroism spectra. The aqueous solution of the polymer exhibits two distinct positive bands preceeding a negative one. The first positive band at 285 nm gives a value of $[\theta]$ of 8300 and the second, at 257 nm, a value of $[\theta]$ of 16,265. In glycol, there is a reduction of the $[\theta]$ values at the two maxima to 3250 and 13,650, respectively, with both bands showing a shift to longer wavelength by 5 nm. In neither of the two solvents does the spectrum bear any resemblance to that of the monomers. In fact, the spectra of the latter are completely nonconservative and suggest an almost total absence of interaction. In addition, while the polymers exhibit one broad positive absorption band with a maximum at approximately 260 nm (with a shoulder at ≅280 nm in glycol), the spectra of the monomeric mixtures are resolved into two approximately equal peaks due to the individual monomers (dCMP at 272 nm and dGMP at 253 nm).

Furthermore, the absorbance of the polymer is noticeably hypochromic relative to the monomers throughout its absorption band in either solvent, another indication of a high degree of interaction between nucleotides in the polymer as compared to the monomer mixture. Finally, the polymer appears to be hyperchromic in the long-wavelength region around 300 nm, an observation which can again be explained in terms of an $n \rightarrow \pi^*$ transition characteristic of highly ordered structures. This assignment is confirmed by the absence of parallelism in the response of the two long-wavelength circular dichroism bands to the change in solvent.

Comparisons of Ribo- and Deoxyribopolymers. Four types of forces currently thought to be present in polynucleotides and capable of determining their conformation are (Felsenfeld and Miles, 1967; Davies, 1967): (a) hydrophobic bonding between bases along the axis of an incipient helix; (b) hydrogen bonding between bases roughly at right angles to this axis; (c) hydrogen bonding between a 2′-OH of the sugar to either a base or a phosphate of an adjacent residue; and (d) electrostatic interactions between adjacent bases. Comparison of deoxyribo and ribo analogs permits an assessment of the contributions due to part c.

Such a comparison for $(dA)_n$-neutral and $(rA)_n$-neutral (Green and Mahler, 1970) indicate the following; the thermal data (Table II) for the aqueous solutions show $(dA)_n$ to be somewhat more stable (T_m) and cooperative ($\sigma_{2/3}$) than $(rA)_n$, but with very similar values for their hyperchromicity. The magnitude of the first extrema in optical rotatory dispersion and circular dichroism for $(rA)_n$ are significantly larger than those for $(dA)_n$. Therefore stacking interactions in the two polymers make very similar contributions to polymer stability in aqueous solution. However, since their rotational properties are different, so must be details of polymer geometry. These differences do not need to be any more profound than those already present in the corresponding dinucleoside phosphates (Warshaw and Cantor, 1970).

An additional difference emerges from a comparison of their properties in glycol. As discussed earlier, the absorption spectra of $(dA)_n$ in water and glycol are similar, while that of $(rA)_n$ is closer to that of the monomer. Similar considerations govern their rotational properties: the rotation and ellipticity of $(rA)_n$ is reduced virtually to the monomer value while $(dA)_n$ retains the characteristic values already described.

Thus, in polyadenylic acids, not only is there no indication of additional helix forming or stabilizing features due to the substitution of the 2′-H by 2′-OH but on the contrary the 2′-OH seems to lead to a reduction in magnitude of parameters measuring stabilization or optical dissymmetry.

Another interesting comparison can be made between $(rU)_n$ (Green and Mahler, 1970) and $(dT)_n$. The reduction in magnitude of the first Cotton effect, and of the positive circular dichroism band, for either polymer, on transfer from water to glycol, is practically identical: the decreases of $[m']$ are to 63 and 61% for $(rU)_n$ and $(dT)_n$, respectively. This close similarity again implies a relative lack of importance of intramolecular hydrogen bonding involving the 2′-OH group, in stabilizing this type of polymer: denaturation by exposure to a solvent of low dielectric constant would be expected to be facilitated in the case of the deoxypolymer and retarded in the case of the ribo analog by the attendant strengthening of such interactions. The same reasoning also applies to the hypothesis of Ts'o *et al.* (1966) which assigns an important role to an *intra-*

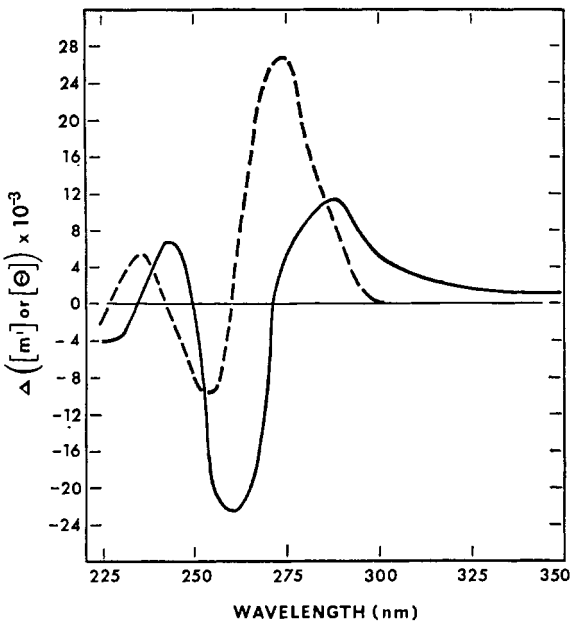

FIGURE 13: Optical rotatory dispersion and circular dichroism difference spectra of $(rG)_n \cdot (rC)_n$ and $(dG)_n \cdot (dC)_n$ in aqueous solution, 0.1 M NaCl–0.01 M sodium cacodylate (pH 7.0). Optical rotatory dispersion (——) and circular dichroism (-----) difference spectra plotted as $(rG)_n \cdot (rC)_n - (dG)_n \cdot (dC)_n$.

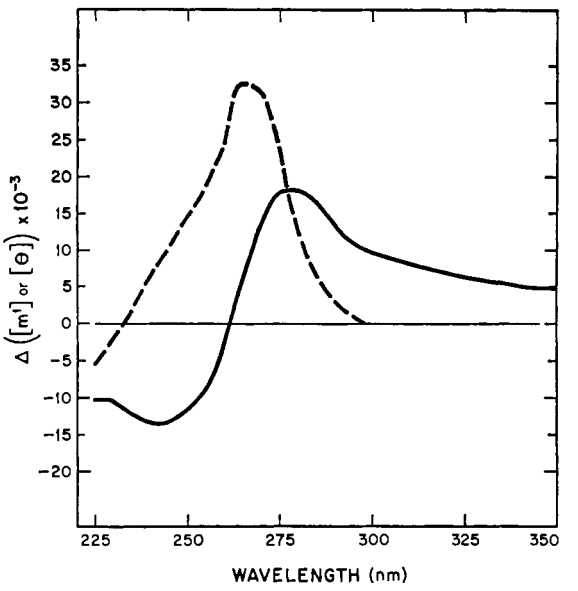

FIGURE 14: Optical rotatory dispersion and circular dichroism difference spectra of $(rA)_n \cdot (rU)_n$ and $(dA)_n \cdot (dT)_n$ in aqueous solution, 0.05 M KF–0.001 M EDTA (pH 5.8). Optical rotatory dispersion (——) and circular dichroism (-----) difference spectra plotted as $(rA)_n \cdot (rU)_n - (dA)_n \cdot (dT)_n$.

molecular hydrogen bond between a 2′-OH and the 2-keto group of the uracil in $(rU)_n$.

Figure 13 shows a plot of the optical rotatory dispersion and circular dichroism difference spectra between $(rG)_n \cdot (rC)_n$ and $(dG)_n \cdot (dC)_n$ in aqueous solution in order to assess the transitions affected by substituting a 2′-OH group in the case of a duplex between two different homopolymers. It is apparent that this substitution leads to profound differences. In particular, the optical rotatory dispersion spectrum shows difference maxima at 287, 260, and 245 nm, while the circular dichroism spectrum exhibits difference maxima at 274, 252, and 235 nm (crossover at 258 nm). These differences are very similar to those that can be calculated for the corresponding homo- and heterodimers from the data of Warshaw and Cantor (1970). The principal electronic transition responsible is probably a $\pi \rightarrow \pi^*$ excitation with an absorption of $\cong$255 nm, *i.e.*, the absorption maximum of the polymer, and responsible for two symmetrical (*i.e.*, conservative) circular dichroism bands: a negative one centered at 252 nm and a positive component centered at about 275 nm and contributing significantly to the long-wavelength band. The latter appears to be composed of two bands with an additional major contribution by a (probably nonconservative) band centered at $\cong$280 nm. This satellite band is in the region where one would expect to see an $n \rightarrow \pi^*$ transition (a discussion of possible $n \rightarrow \pi^*$ transitions in $(rG)_n$ is presented in Green and Mahler, 1970). Thus the substitution of a 2′-OH for H in the sugar produces a change in conformational fine structure reflected by an increased interaction between transition moments polarized both parallel and at right angles to the helix axis. It is, however, not possible to be more precise as to whether the observed change reflects a primary effect in the sense of the substitution actually leading to a structure containing certain new bonds not present in its absence, or of simply permitting certain new or enhanced electronic interactions within an identical structural framework.

Figure 14 was plotted in the same manner as Figure 13, to compare the two duplex polymers, $(rA)_n \cdot (rU)_n$ and $(dA)_n \cdot (dT)_n$ in aqueous solution. Again, as was seen previously, substitution of a 2′-OH in the polymer was found to alter the helix geometry [since thymine and uracil have roughly the same electronic properties, this base difference is expected to make minor contributions only (Langridge and Marmur, 1965)]. Interestingly enough, in this instance only the $\pi \rightarrow \pi^*$ transitions appear to be affected.

In the optical rotatory dispersion difference spectrum, only two extrema are observed: a peak at 278 nm with an $[m']$ value of 18,400 and a trough at 243 nm with an $[m']$ value of 13,750 (crossover at 262 nm). The optical rotatory dispersion difference spectrum, thus, appears to be centered in a region where only $\pi \rightarrow \pi^*$ transitions occur. The circular dichroism

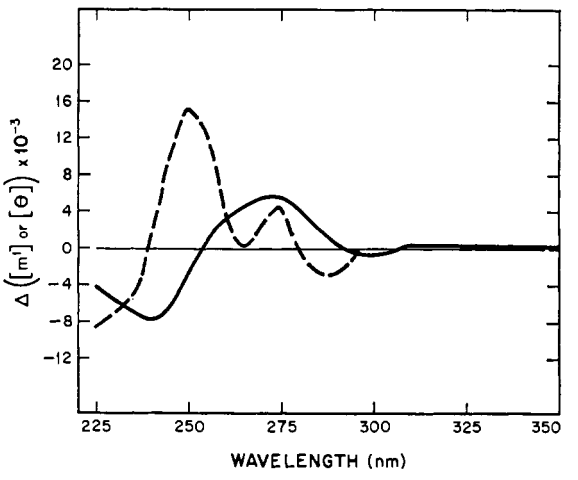

FIGURE 15: Optical rotatory dispersion and circular dichroism difference spectra of $(dAdT)_n \cdot (dAdT)_n$ and $(dA)_n \cdot (dT)_n$ in aqueous solution, 0.05 M KF–0.001 M EDTA (pH 5.8). Optical rotatory dispersion (——) and circular dichroism (-----) difference spectra plotted as $(dAdT)_n \cdot (dAdT)_n - (dA)_n \cdot (dT)_n$.

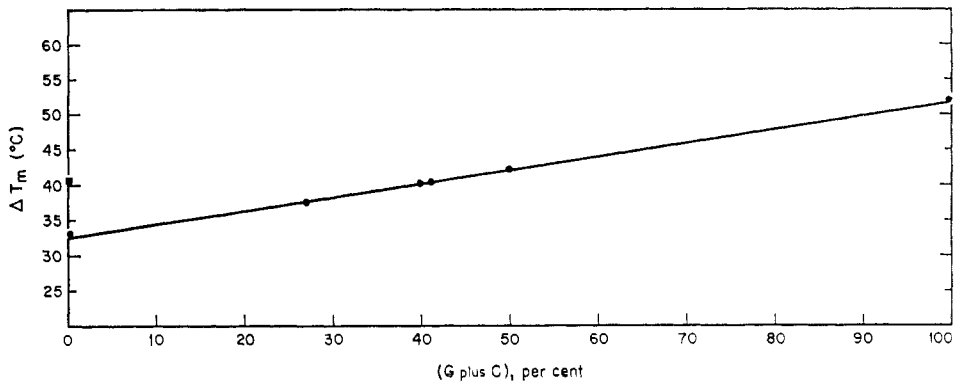

FIGURE 16: Plot of ΔT_m (T_m (water) − T_m (glycol) *vs.* the percentage of guanine plus cytosine in the deoxypolymer. T_m's were recorded in 0.05 M KF–0.001 M EDTA (pH 5.8). The following polymers were used: 0% G plus C — $(dA)_n \cdot (dT)_n$; [$(dAdT)_n$], is also shown for comparison (□), 27% G plus C — *C. perfringens* DNA, 41% G plus C — salmon sperm DNA, 42% G plus C — calf thymus DNA, 50% G plus C — *E. coli* K-12 DNA, 100% G plus C — $(dG)_n \cdot (dC)_n$.

difference spectrum can be interpreted in an analogous fashion. Only one very intense band with a $[\theta]$ of 32,700 appears in the region under investigation (at 265 nm). This band is nonsymmetrical with a definite skewness on the short-wavelength side and thus is composed of, at least, two transitions, both of $\pi \rightarrow \pi^*$ origin. Since the band is Gaussian on the long-wavelength side, it is logical to conclude that the $n \rightarrow \pi^*$ transitions, usually seen in the region $\cong$280 nm, are either absent or are of equal magnitude in the two polymers.

Thus optical rotatory dispersion or circular dichroism difference spectra provide for differences in the geomtry of the two double-stranded helices presumably caused by the presence of a 2′-OH in one of the polymers. As a result of this substitution the bases become oriented in a manner so as to affect their interaction at right angles to the helix axis without any effect on any contribution parallel to this axis.

Figure 15 shows the optical rotatory dispersion and circular dichroism difference spectra between the two adenine and thymine containing deoxypolymers, $(dAdT)_n \cdot (dAdT)_n$ and $(dA)_n \cdot (dT)_n$. The spectra are quite complex, but it appears that both the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions are affected. The optical rotatory dispersion curve shows three extrema: a very low intensity trough centered at 299 nm, a peak at 273 nm and a second trough at 240 nm. The circular dichroism spectrum shows three distinct bands and the beginnings of a fourth one. The long-wavelength circular dichroism band has its center at 287 nm, that is, in a region which is associated with the $n \rightarrow \pi^*$ transition. The other two bands, centered at 250 and 274 nm, are related to the $\pi \rightarrow \pi^*$ transitions.

The spectra provide clear indication for differences between the two polymers as far as their helix geometry in solution is concerned. This confirms and extends conclusions based on X-ray diffraction studies on their solid fibers (Davies and Baldwin, 1963; Langridge, 1966).

Thermal Measurements. All thermal measurements for the deoxypolynucleotides are presented in Table II. It is interesting to note that only heteropolymer duplexes were sufficiently stable to show melting in glycol solution. This was previously observed with the ribopolymers where $(rA)_n \cdot (rU)_n$ and $(rG)_n \cdot (rC)_n$ exhibited melting transitions while the acidic forms of $(rA)_n$ and $(rC)_n$ did not (Green and Mahler, 1970). It can also be observed from Table II that the $\sigma_{2/3}$ values for the polymers in water and glycol are practically identical. This is an indication that the cooperativity of the helix–coil transition is the same in the two solvents, that is, all regions in the polymer are equally susceptible to interaction with the solvent and no new regions of different thermal stability are introduced by selective interaction with it.

Figure 16 shows a plot of ΔT_m [T_m (abs) in water minus T_m (abs) in glycol] *vs.* the mole fraction (G + C) in the polymer. Values for T_m were taken from Tables II and V. The polymers used for 0 and 100% (G + C) were $(dA)_n \cdot (dT)_n$ and $(dG)_n \cdot (dC)_n$, respectively. The interesting result here is that the correlation remains linear throughout, even at the extremes where one would expect to see deviations. The correlation of (G + C) content with T_m in aqueous solution is well established, but suffers from such a lack of linearity when the (G + C) content of the polymer reaches extreme values.

It should be mentioned that the two duplex polymers, $(dAdT)_n \cdot (dAdT)_n$ and $(dA)_n \cdot (dT)_n$ gave different ΔT_m's. It was the former that deviated sharply from the linear relationship and this was not unexpected since other laboratories found analogous deviations of other parameters for this polymer, *i.e.*, for optical rotatory dispersion (peak at 290 nm) (Samejima and Yang, 1965); T_m (Marmur and Doty, 1959, 1962) and circular dichroism (Bernardi and Timasheff, 1970). Apparently, although the alternation of bases in this duplex produces a more regular and DNA-like helix in the fiber than is the case for the isomer with all the purines in one strand and all the pyrimidines in the other, interaction with nearest neighbors appears to be stronger in the latter case since $(dAdT)_n \cdot (dAdT)_n$ is more susceptible to glycol denaturation and has a lower T_m in glycol than does $(dA)_n \cdot (dT)_n$ (see Table II).

Conclusions

Certain conclusions concerning the structure of DNA in glycol appear warranted from the data presented in this and previous papers (Green and Mahler, 1968, 1970). They are: (a) the structure exhibits considerable secondary order and is not identical with that of denatured DNA in aqueous solution —or to that of the component nucleotides. (b) The structure cannot be *identical* with the Watson–Crick–Wilkins "B form" of DNA. This conclusion is based on the decidely different optical rotatory dispersion and circular dichroism spectra for DNA obtained in aqueous and glycol solutions. (c) The form DNA assumes in glycol is reversible, that is dialysis *vs.* increasing concentrations of water regenerates the original native aqueous form of DNA, as measured by optical rotatory dispersion, circular dichroism, or T_m. This allows one to conclude that the strands have never separated and have remained in register even though the external environment has changed

from an aqueous to a nonaqueous one. (d) The structure is probably double stranded. DNA in glycol exhibits a highly cooperative thermal transition ($\sigma_{2/3}$ equals 4.5°) with the high hyperchromicity of 39%. Both values are characteristic of double-stranded, as opposed to single-stranded, DNA. And (e) the mass/unit length for the aqueous and glycolic forms of DNA are very nearly identical (Luzzati *et al.*, 1964).

While this manuscript was in preparation Nelson and Johnson (1970) published a note which confirms all the essentials of our observations on the nature of the circular dichroism spectrum of calf thymus DNA in ethylene glycol.[2] They, as we, were struck by the similarity of these spectra with ones reported by Tunis and Hearst (1968) for DNA in concentrated salt solution; and of our optical rotatory dispersion spectra with those of the DNA in bacteriophage capsids published by Maestre and Tinoco (1965, 1967). On the basis of having available previously unpublished results by Tunis-Schneider and Maestre (1970) on unoriented DNA films under a variety of environmental conditions Nelson and Johnson were able to conclude that the novel conformation assumed by DNA in glycol, and approached in high salt, corresponded to the C form (Marvin *et al.*, 1961) with 9.33 base pairs/turn, 13-Å pitch, and a 6° tilt. This assignment appears to be consistent with all the other details described above.

The ability to assume the C conformation in glycol appears to be a general property of all DNAs tested regardless of base composition, as shown by their capability of undergoing gradual and strictly reversible transition from the aqueous (B) to the glycol (C) form as a function of solvent composition. All of them are also capable of undergoing a highly cooperative thermal transition in glycol to a conformation reminiscent of, but not identical with, that assumed in water at elevated temperatures (Mahler *et al.*, 1968) quite different from that of a mixture of the various nucleotides (Figures 4 and 6) and indicative of the retention of considerable short range order. It can therefore not correspond to that of a true random coil in this solvent.

A comparison of DNAs with synthetic homo- and heteropolynucleotides discloses significant differences. In the deoxyribo series—unlike their ribo analogs—all complementary duplexes appear to retain this basic structure and are still capable of undergoing thermal transitions in glycol analogous to those observed in aqueous solution, with G·C base pairs—or at least the distribution of G + C—providing additional incremental stability (Figure 16). However, only the *adenine*-containing polymers of the *deoxy series* exhibit some or all of the characteristic optical features of DNA in this solvent. We are therefore led to the conclusion that it is the presence of deoxyadenosine *itself* (in conjunction perhaps with neighboring thymines on the same strand) in a polymer that permits it to assume the geometry characteristic of the C conformation. The strongest confirmatory evidence for this assertion is provided by an examination of the circular dichroism spectra characterizing the low-temperature form of $(dAdT)_n \cdot (dAdT)_n$ in glycol. Comparison to the data published by Nelson and Johnson[2] shows an overall similarity of fine structure at long wavelengths with the diagnostic pattern of a negative, conservative transition in this region [coming in the duplex $(dAdT)_n$, however, at shorter wavelength (270 nm rather than 290 nm) and with greater magnitude relative to the principal negative circular dichroism band than in DNA and hence probably referrable to a $\pi \rightarrow \pi^*$ rather than an $n \rightarrow \pi^*$ electronic transition]. If, therefore, the alternating copolymer duplex retains its B conformation in aqueous solution, as seems likely from its spectra, then the spectra shown in Figure 10 may be those characteristic of this duplex in a conformation analogous to but not identical with the C conformation of DNA. It should also be reemphasized that *none* of the ribopolymer duplexes appears capable of assuming this structure in glycol solution.

Finally it appears appropriate to point out that glycol seems to be a rather unique solvent in favoring a reversible transition between the B and the C conformation. While reversible transitions between different conformations are induced when water is replaced by a variety of organic solvents—including primary alcohols—in the presence of electrolytes (Geiduschek and Holtzer, 1958; Helmkamp and Ts'o, 1961; Brahms and Mommaerts, 1964) they differ from the one described here in two important respects: (a) they occur rather discontinuously over a very narrow range of solvent concentration and (b) they lead to forms which differ fundamentally in their rotational properties: the low-temperature forms in the case both of formamide (Ts'o and Helmkamp, 1961) and of ethanol (Brahms and Mommaerts, 1964) are ones that show a relatively high rotation or dichroism in the long-wavelength region.

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Magnesium-Induced Conformational Change in Transfer Ribonucleic Acid as Measured by Circular Dichroism*

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ABSTRACT: The circular dichroism spectra of purified $tRNA^{fMet}$, $tRNA_2^{Phe}$, $tRNA^{Val}$, $tRNA^{Arg}$, and $tRNA^{Glu}$ from *Escherichia coli*, and also unfractionated tRNA from *E. coli*, have been examined in the region 200–380 nm. It was found that a minimum centered at about 210 nm showed an increase in magnitude of 20–28% on the addition of 10 mM magnesium chloride to a solution with an ionic strength of 0.2 M and a pH of 7.0. The 260-nm maximum showed an increase only 5–12% in magnitude. The region above 300 nm, primarily due to the presence of 4-thiouracil in many *E. coli* tRNAs, gave a variety of spectra, and the effect of adding Mg^{2+} was also very variable. However, all of the measured changes occurred in a concerted manner, supporting the idea that the binding of a small number of magnesium ions gives rise to a specific conformational change in the tRNA. The data, treated in terms of a two-state hypothesis, were consistent with the idea that the binding of three magnesium ions, with an average binding constant of 3.2×10^2 M^{-1} is required for the conformational charge. $tRNA^{Glu}$ was unusual in that it showed little absorption in the 4-thiouracil region, but had a strong negative circular dichroism in this same region. Also it did not have the 295-nm minimum found in all other tRNAs studied.

The affinity of magnesium ion for polynucleotides and its stabilization of their secondary structure is well known (Felsenfeld and Miles, 1967). Considerable work has also implicated Mg^{2+} as a necessary requirement for the correct secondary and tertiary structure of tRNA (Adams *et al.*, 1967; Ishida and Sueoka, 1967; Reeves *et al.*, 1970), and its reactivity in the aminoacylation reaction (Adams *et al.*, 1967). These latter authors have reported that metastable states of tRNA can be created under certain conditions. These states are inactive with respect to the aminoacylation reaction, which includes 5–10 mM Mg^{2+} in the assay system, but can be reactivated by heat treatment in the presence of Mg^{2+}. It has been shown that there are concomitant changes in the physical parameters of tRNA, and thus it has been suggested that Mg^{2+} is a necessary requirement for the biological activity of tRNA (Lindahl *et al.*, 1966; Adams *et al.*, 1967; Ishida and Sueoka, 1967).

On the other hand, experiments have been performed that suggest that Mg^{2+} (or any other divalent ion) is not a necessary requirement for the biological activity of tRNA, at least insofar as the aminoacylation reaction is concerned. Lagerkvist *et al.* (1966) have shown that $tRNA^{Val}$ can be charged by Val-AMP in the absence of Mg^{2+}. Also, a later communication by Ishida and Sueoka (1968) has indicated that an inactivated state of tRNA can be reactivated in the absence of Mg^{2+} by using high concentrations of monovalent salt.

Manganous ion substitutes for Mg^{2+} in reactions involving tRNA. The paramagnetic properties of Mn^{2+} have been useful in studying its binding to nucleic acids. Studies of the enhancement of the proton relaxation rate of water by Mn^{2+} (Cohn *et al.*, 1969) and of the free Mn^{2+} concentration as measured by electron spin resonance (Danchin and Guéron, 1970) suggested that there is a class of interacting binding sites in tRNA, which is not observed in synthetic polymers, such as poly(A). This cooperativity has been presumed to reflect a conformational change in the tRNA (Danchin and Guéron, 1970).

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